New approaches for brain repair –

from rescue to reprogramming

Roger A Barker[1], Magdalena Götz[2,3], Malin Parmar[4]

[1] Department of Clinical Neuroscience and Cambridge Stem Cell Institute, University of Cambridge, Cambridge CB2 0PY

[2] Institute for Stem Cell Research, Helmholtz Center Munich and Physiological Genomics, Biomedical Center, Ludwig-Maximilians University, Großhadernerstr. 9, 82152 Planegg-Martinsried, Germany

[3] SyNergy, Excellence Cluster Systems Neurology, Center for Stroke and Dementia, Feodor-Lynenstr.17, 81377 Munich, Germany

[4] Wallenberg Neuroscience Center and Lund Stem Cell Center, Lund University, BMC A11, Sölvegatan 17, S-221 84 Lund, Sweden

Email: rab46@cam.ac.uk; Tel: +44 1223 331160; Fax: +44 1223 331174

Email: Magdalena.goetz@helmholtz-muenchen.de

Email: malin.parmar@med.lu.se

Acknowledgements and Funding

Roger Barker is funded by the NIHR Biomedical Research Centre in Cambridge, Cure PD, PDUK, European Research Council under the European Union's Seventh Framework Programme: FP/2007-2013 NeuroStemcellRepair (no. 602278). Wellcome Trust MRC Stem Cell Institute and MRC UKRMP PSCP. He has received consultancy payment from FCDI and LCT.

MG is funded by the German research foundation (CRC870, SPP1738, 1757, EXC1010 Synergy), The Ministry of Science and Research (MAIV), ERANET and the ERC (ChroNeuroRepair). Patent WO 2015/114059 A1.

MP receives funding from the New York Stem Cell Foundation, the European Research Council under the European Union's Seventh Framework Programme: FP/2007-2013 NeuroStemcellRepair (no. 602278) and ERC Grant Agreement no. 30971, the Swedish Research Council and the Strategic Research Area Multipark at Lund University Multipark. MP is a New York Stem Cell foundation Robertson Investigator. MP is the owner of Parmar Cells and co-inventor of patent 62/145,467.

We would like to thank Danielle Daft for her help in the preparation of this manuscript.

PREFACE

The ability to repair or promote regeneration within the adult human brain has been envisioned for decades. However, to date the results have largely been disappointing and typically involved delivery of growth factors and cell transplants designed to rescue or replace a specific population of neurons. Of late, new approaches using stem cell derived cell products and direct cell reprogramming have opened up the possibility of effecting better repair with the reconstruction of neural circuits. In this review we briefly summarise the history of neural repair and then discuss these new therapeutic approaches especially with respect to chronic neurodegenerative disorders.

INTRODUCTION

The mammalian central nervous system (CNS), unlike many other organs, has a limited capacity for self-repair and our ability to overcome this would transform how we could treat patients with a vast array of neurological disorders. However, limited neurogenesis in the adult CNS, the failure of axonal regeneration and the non-permissive local environment have been major barriers, literally, for CNS repair. Nevertheless, in recent years it has been shown that much can actually be done in the mature CNS to foster innate regeneration.

Diseases or disorders of the CNS can be developmental, inherited or acquired; acute or chronic and each brings with them a different set of challenges when developing repair strategies. Both acute damage and chronic neurodegenerative disorders (NDDs) are characterised by an inter-related macroglial and microglial/inflammatory reaction with changes in the extracellular matrix and/or alterations in the blood brain barrier. Therefore, agents improving this environment could be used to help protect neurons from further damage and promote circuit reformation and function. Such disease modifying therapies using, for example, trophic factors or cell transplants releasing such factors could in theory be used either alone at early stages of disease or combined with approaches designed to replace lost cells through either cell transplantation, re-activation of endogenous neurogenesis, or directed reprogramming in situ. In this review we will discuss three main strategies for restoring function in the damaged/diseased CNS:

(i) cell rescue using neurotrophic factors or cell-based approaches that employ putative disease modifying effects;
(ii) cell replacement therapies using transplants of exogenously derived and in vitro cultured cells;
(iii) cell replacement using endogenous neural precursor cells or strategies that involve the direct reprogramming of resident cells within the brain.

FIGURE 1 here

NEUROTROPHIC FACTORS and DISEASE MODIFICATION

Enhancing the regenerative potential of the CNS in the face of a disease process can be done in 2 major ways. Firstly using approaches that restore and maintain the functional integrity of those cells that are dysfunctional but not lost. This typically involves neurotrophic factors, some of which could be delivered using cell-based treatments. However grafted cells could also be used to protect dysfunctional cells from ongoing insults such as metabolic stress. Alternatively it could be done through minimizing environmental barriers to repair, e.g. by modifying the extracellular matrix and/or glial/immune reaction (see Adams and Gallo, 2018[1]; Fawcett 2015[2]).

The ability to promote endogenous repair using exercise based therapies as well as cognitive training has been shown experimentally to have effects on promoting endogenous neurogenesis and the local production of growth factors such as BDNF in the brain[3]. However, whether these changes seen in experimental animals can translate into clinical benefits is largely unknown but such intervention do have the potential to be used in combination with cell replacement/rescue therapies to optimise reparative benefits.

The use of trophic factors has been explored for over 20 years, most notably in ALS and PD (reviewed in Bartus & Johnson, 2016[4,5]). These initial studies suffered from major problems with delivery – the factors either failing to reach their neuronal target or being rapidly inactivated. This led to a new round of trials with intraparenchymal delivery, most notably with GDNF delivered into the striatum of patients with moderately advanced PD. In two open label studies, from 2 independent centres, this produced positive clinical responses with post mortem and imaging data supporting its mode of action[6-9]. These results led to a double-blind placebo-controlled trial of 34 patients with PD, which found that patients in receipt of GDNF showed no significant benefits at 6 months[10].

The reasons for the discordant results from these trials have been discussed extensively (e.g.[11]), including placebo effects, although this would not explain the reported changes on PET imaging and at post mortem in patients treated with GDNF infusions. Furthermore, in the double-blind study, the group in receipt of saline did not improve which also argues against any placebo effects and suggests that the trial failed because the active treatment did not work. This could have been due to the dose of GDNF and/or the way it was delivered, both of which were different to those employed in the open label studies. Given this uncertainty, further trials have been undertaken including a double-blind approach with a new convection enhanced delivery system that better delivers GDNF. However, so far positive outcomes have not been achieved (https://scienceofparkinsons.com/2016/07/07/the-gdnf-trial-bristol-initial-results/) which suggests that the use of trophic factors alone may not work to treat such chronic NDDs. This is reinforced by parallel studies delivering other GDNF like factors [12] including Neurturin using an AAV delivery system (AAV-NTN). Here, the initial open label studies showed efficacy but 2 subsequent double-blind placebo control studies (which differed in terms of where, and how, the AAV-NTN was delivered) failed to show efficacy[13-15].

So, does this mean that repairing the brain with trophic factors is not a viable way forward? This is likely to be the case when the disease has advanced beyond a certain stage- namely once you have lost a critical number of cells/fibres, it is unrealistic to expect any growth factor to work, as there is nothing left for it to regenerate! Given that most NDDs present clinically when there is already extensive cell/fibre loss, the early use of these agents will be required- and a post hoc analysis of some of the trial data with GDNF and AAV-NTN supports this view[14,15]. Moreover, the disease state may also interfere with growth factor function. For example GDNF may not be able to act through its normal receptor in the presence of alpha synuclein pathology in PD because of alterations in Nurr1 signalling, which is needed for this trophic factor to produce its therapeutic effect[16]. Thus, it may be necessary to enhance Nurr1 levels ahead of using GDNF in PD for it to work efficiently, necessitating the adoption of combination approaches in any trials going forward. Finally, the optimal dose of any growth factor and how to best deliver it, including the volume of distribution needed to maximise its benefits, are still major issues to solve. To date, GDNF and AAV-NTN have been delivered in ways that are insufficient to cover the structure being treated, and thus many of the failures seen clinically may relate to this- for which there is some support from post mortem data relating to both the original GDNF and subsequent AAV-NTN studies[9,15].

Overcoming environmental hurdles to regeneration

Promoting neuronal survival and outgrowth is only one aspect of the problem in repairing the CNS. The other is enhancing the environment to promote that regenerative process. This essentially distils

down to modifying the glial elements and/or the ECM, both of which have been reported to provide inhibitory signals for repair in the adult CNS[1,2].

In terms of the glial cells, it has long been known that astrocytes and oligodendrocytes both express cell surface inhibitors to the regenerating axon, although the expression and extent of these varies as a function of disease state- e.g. greater with acute traumatic injuries than chronic NDD. This premise that glial cells produce inhibitory factors which can be blocked has led to early clinical trials most notably using anti-NOGO antibodies in spinal cord injury (http://clinicaltrials.gov/show/NCT 00406016). However, this concept that the glia are "bad" for CNS repair has been challenged, in that the glial scar/reaction to injury or disease involves a heterogeneous collection of cells, which have a variety of functions. So, under some conditions the glial response may be beneficial for repair, possibly through effects on the inflammatory response to that CNS disease[1]. Conversely under other circumstances, the opposite may occur. For example, it has recently been reported that microglia can induce astrocytes to become neurotoxic[17]. This concept of 'gliopathy' is not new and has been explored in ALS where transplanted glial cells have been used to better buffer extracellular glutamate and by so doing protect motorneurons[18,19]. Whether such an approach could be used for treating ALS clinically is unknown but highlights the potential use of non-neuronal cell transplants to protect the neurons subject to disease.

More recently there has been interest in the ECM of the brain and especially peri-neuronal nets (PNNs). These latter structures are unevenly distributed across the brain and spinal cord and appear to have many functions during development and in the adult brain including a role in neural plasticity. This has been shown to extend to NDDs, most notably in transgenic models of AD, where removing PNNs using either Chrondoitinase-ABC (Ch-ABC) or genetically, has been shown to improve cognitive function while not influencing the underlying disease process-possibly by enhancing neurite outgrowth and synaptic plasticty[20],[21]. All of which suggests that the use of this, or similar, agents may have benefits when combined with cell therapies in which synaptic integration with host cells may be restricted by local PNNs and/or disease driven increased expression of inhibitory ECM molecules.

REPLACEMENT THERAPIES USING EXOGENOUS CELLS

When a NDD or injury reaches the point where a critical number of neurons have been lost, the replacement of these neurons is essential for functional improvement. This can be done through replacing the lost cells by transplantation into single or multiple brain regions to partially recreate complex circuits either locally or over long distance as outlined in FIGURE 2.

Figure 2 here

For this to work, a number of important questions need to be addressed. Firstly what is the optimal cell type(s) to be transplanted and at what developmental stage should it be harvested for grafting? Secondly what CNS location(s) is/are needed for optimal recovery and fibre outgrowth. Thirdly how many cells are needed for effective repair and finally should other interventions be done either at the same time as grafting or post transplantation to enhance survival, integration or function.

Cell Replacement Therapy using human fetal allografts

PD is one of the least complex NDD to target with cell-based therapies given the fact that the local degeneration of DAergic neurons in the midbrain is known to be critical to the clinical expression of

many features of this disorder. It has therefore been the subject of a relatively large number of clinical transplantation trials using the developing midbrain dopamine cells derived from the human fetal ventral midbrain (VM)[22]. Although the outcome of these trials has been highly variable[23], they have shown that at least a sub-group of PD patients in receipt of such transplants from different centers do well[24-27]. However, it has also been shown that over time, a minority of the transplanted cells become affected by the disease process and show protein aggregation[28,29], and that some patients can experience troublesome side effects such as graft induced dyskinesias[30-32] relating to graft composition and their capacity to innervate the whole putamen[22].

Clinical trials using transplants of primary human fetal striatal tissue have also been performed in HD patients[33-35] on the premise that in HD the medium spiny neurons (MSN) of the striatum, are the ones that are primarily lost[36]. In this case, the neurons are placed homotopically into the striatum to reconstruct basal ganglia circuits. To date the effects seen in patients are modest at best [33-35], with evidence for poor graft survival and integration[37].

Other NDDs and CNS disorders have a more complex pathology, involving several cell types and/or cells in many regions of the CNS and it is hard to see how these could be treated by fetal tissue transplants, although this may be different with stem cells given our ability to differentiate them to a wide range of neuronal phenotypes.

Cell Replacement Therapy using stem cells

A major challenge for developing any cell replacement therapy is the ability to reliably and robustly generate large numbers of relevant cells. Recent developments using human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) has now made this possible[38] with clinical trials imminent using this approach in PD, for example [39,40].

Additionally, stem cell derived DA neurons have also been used to better understand graft function, innervation and integration in pre-clinical animal models of PD. For example, comparative studies with human fetal VM tissue have shown that both hESC and hiPSC-derived DA progenitors are very similar to their fetal counterparts[41,42] mediating functional recovery with equivalent potency over a similar time course[43]. Coupled with new methodologies for assessing graft function and integration, their mechanism of action, as well as their ability to appropriately innervate and integrate into host circuitry have also been studied. These studies have shown that DA neurons transplanted to the rat brain can innervate correct target structures when placed either locally at the site of action in the striatum or back in the midbrain which requires more extensive axonal outgrowth from the cells[42,43]. Furthermore, they not only innervate but integrate into host neuronal circuitry[44] with functional recovery [38,45]. Similarly, transplantation of stem cell derived MSNs have now been shown to survive and function in rodent models of striatal degeneration (used to model HD)[46-48].

Another advantage of using stem cells derived transplants is the ability to remove or minimize unwanted neuronal subtypes, e.g. serotonergic neurons in PD, that may mediate the graft-induced dyskinesias seen with fetal human VM transplants. Furthermore, the effect of grafting pure populations of neurons, or a combination of neuronal and or astrocytic cell types can be compared. Indeed deficient astrocyte function may contribute to the loss of neurons in many NDDs and the absence of such cells may impact on neuronal survival in the transplant- as has been shown in fetal striatal grafts in patients with HD[49]. As an alternative to the possible co-grafting with glial cells, other agents or biomaterials engineered to express growth factors, immune modulating components or similar to provide a supportive and protective microenvironment could be delivered together with the neurons and this may even include some form of scaffolds for larger lesions such as stroke [50].

Genetic modifications or correction of the cells prior to transplantation could also be used to make the grafted cells resistant or refractory to the disease pathology. This may be of critical importance when the transplants are derived from the patients' own cells[42], or in diseases such as ALS where the motor neuron loss is driven in part by astrocytes. However, even unrelated healthy donor cells can be affected by proteinopathies in some NDDs, as has been seen in the PD and HD fetal transplant trials[28,29,51]. In these cases, making cells resistant to the disease by removing the normal protein onto which the pathological species templates could be favourable.

Additionally, as genetic engineering tools become more refined, stem cells could be easily modified to enhance aspects of their behaviour, such as modifying chemotactic interactions between the grafted cells and their progeny to enhance migration[52]; making them refractory to the inhibitory cues of the glial scar; increasing their innervation potential by enhanced expression of polysialic acid [53] (all of which would need to done so that the enhanced outgrowth is targeted specifically to the area for repair) and/or decreasing their immunogenicity[54,55]. Based on cell transplants in animal models, the possibility of engineering stem cell progeny so that their function can be modified using opto- and chemogenetic interventions is now also being considered [45,56,57].

Cell Replacement therapy for circuit reconstruction

Due to the complexity of the organization of the cerebral cortex, replacement of lost neurons is a more challenging task, and is thus still at the pre-clinical stage. New technologies for studying cell integration and function have shown that transplanted cells mature, integrate into local host circuitry, form functional synapses, mediate recovery and provide circuit repair in animal models of AD, stroke and cortical lesions[58-62]. Importantly, it has recently been shown that the exact brain-wide input connectome of neurons in the murine visual cortex can be replicated by transplanted neurons[59]. This is important as aberrant connectivity could lead to deleterious consequences such as epileptic seizures. Thus, the adult mammalian brain is more plastic than previously thought in terms of accommodating new neurons and building new functional circuitry.

REPLACEMENT THERAPIES USING ENDOGENOUS CELLS

FIGURE 3 here

Brain repair from endogenous sources has been considered for some time[63], especially after the (re-) discovery of adult neurogenesis and proof for its existence in the human brain[64,65], prompting attempts for recruitment of new neurons from endogenous neurogenic niches to achieve repair in the injured brain[66,67] (Figure 3). Simply recruiting new neurons from active neurogenic sites appears intuitively to be the most promising and easiest to achieve. However, in most cases, the subtypes of neurons needed for repair are different from those endogenously generated. Hence, attempts to recruit neuroblasts to form neurons at other sites met with only limited success, with many of the neuroblasts streaming to the injury site but then failing to fully differentiate into appropriate neuronal types which can survive long term[67,68] (Figure 3). However, adult neurogenesis is very species-specific[69] and endogenous striatal neurogenesis that has been said to occur in the human (but not rodent)[70,71] brain may prove useful and more easily manipulated to generate the relevant neuronal subtypes lost in the striatum after stroke or in NDD[72,73].

A further approach to minimize neuronal loss or help repair and replacement is through the recruitment of stem cell derived astrocytes. These differ from the local parenchymal astrocytes and release an array of beneficial growth factors[74]. However, to what extent this also occurs in human patients and can be used for therapy is currently unknown.

Excitingly, the approach to turn these or other parenchymal glial cells into functional neurons by forced expression of neurogenic fate determinants has come a long way surprisingly fast. This was first tested in 2002 in the nervous system, by turning glial cells into neurons in a dish[75]. The first attempts to achieve this in an injured brain in vivo in 2005 were exciting as proof-of-principle experiments, but disappointing in terms of quantitative outcome[76,77,78]. This has been overcome more recently [79-81], and there is now encouraging data on fate conversion of local non-neuronal cells towards the neuronal phenotype of the sort affected by the disease process.

A single neurogenic transcription factor (acting also as potent fate determinant in development) can in many cases be sufficient to convert glial cells into fully functional neurons[82]. For example, Neurogenin2 or NeuroD1 can yield glutamatergic neurons from proliferating glial cells in the adult cerebral cortex including in the highly inflamed brain environment found after TBI[79,83,84]. The key to generating highly efficient neuronal conversion protocols is not necessarily to add more transcription factors, but rather to keep the induced neurons alive by protecting them from death and promoting their maturation. Many neurons induced by direct reprogramming from glial cells die because of the local generation of excessive reactive oxygen species (ROS) – in a process known as ferroptosis [79,84]. By keeping the new neurons alive and reducing ROS levels, efficiencies of over 90% have been achieved, with almost all glial cells, transduced with the neurogenic transcription factor, acquiring a neuronal identity within 1-3 weeks[84]. Importantly, these neurons also acquired a pyramidal neuronal morphology with the identity of deep layer neurons at least at the transcriptional level. Also in other brain regions, this approach has been promising including in the minimally injured striatum using Sox2 and growth factor treatment, the midbrain and the spinal cord[85] and in the cerebral cortex using NeuroD1 and an amyloid plaque deposition model[83]. In these latter approaches, a mix of glutamatergic or GABAergic neurons has been obtained which may be beneficial if several types of neurons need to be replaced or synaptic plasticity increased by providing new GABAergic interneurons as shown through transplantation studies[86,87]. Importantly, synaptic plasticity and new circuit formation using these approaches could also be further promoted by co-administering rehabilitation therapies (see above).

The promising outcome of direct reprogramming approaches raises two important issues, namely which glial cells to best target and which vectors to use. Targeting proliferating glial cells has obvious advantages [88], but they are heterogeneous [1,76,89]. Specific targeting of glial subtypes has been achieved using subtype-specific promoters driving either directly the reprogramming factors[83,90,91] or cell type specific Cre expression in transgenic mice[80,81,85]. Excitingly, this approach has now been shown to work with adeno-associated viral vectors (AAVs) that not only have been used in patients clinically but also can preferentially infect glial cells[92-94]. AAVs have been used to reprogram oligodendrocyte progenitor cells[80,95] to a fate that is dependent on the transcription factor combination- e.g. generating fast spiking parvalbumin+ interneurons in the intact striatum[95]. Moreover, these neurons have been shown to receive input from local interneurons[80]. Finally, recent evidence suggests that striatal and midbrain astrocytes and NG2 glia can be converted to neurons with functional GABAergic and DAergic like phenotypes in a dopamine depletion model[96]. While these pioneering studies show that newly induced neurons can connect locally and result in behavioural changes, their long-distance input and output and contribution to the neuronal network still remains to be explored.

Thus, direct neuronal reprogramming in the adult brain has come a long way in the last few years, but the question still remains as to how these exciting new approaches will translate to the clinic. While there are no major obstacles in turning adult human cells into functioning subtype-specific neurons, as has been shown for example with fibroblasts or pericytes in vitro[97-100], neuronal reprogramming in vivo in the diseased brain has proven more challenging[79]. Thus, investigating differences in the starting cell that affects the outcome of neuronal reprogramming as well as the influences in the local (disease) environment is one of the next tasks to further this approach[79]. Moreover, tools need to be developed for non-invasive reprogramming techniques, either using systemic application of viral vectors as is the case for some AAVs[92-94] or using small molecules that have been shown to be efficient in direct neuronal reprogramming in the dish[79,101].

Direct reprogramming also brings new standards to the field as it is important to compare the induced cell type to the endogenous counterpart that is being replaced or instructed (e.g. iPSCs to ESCs). This can be done using functional assessments and genome-wide expression analysis. This is urgently needed for the field of neuronal replacement, as it is so far unknown how authentic the transplanted or reprogrammed neurons really are, compared to their endogenous counterpart. Raising these standards in basic research and then translating them to the clinic will be the challenge of the future.

CONCLUSION AND FUTURE PERSPECTIVES

A broad research approach will be needed to deliver effective repair strategies to the human brain that combine established approaches with new technologies, and ultimately combined treatment strategies. This will only be possible if we both better understand disease states and the contribution of all cells and the ECM to the process of damage and cell loss, as well as the innate regenerative ability of the adult brain and its capacity to be reprogrammed. By combining approaches to optimize the disease environment and synaptic plasticity while providing new neurons for replacement therapy, new circuits could be rebuilt with functional benefits across a range of disorders eventually developing into a more standard way by which to treat patients. However, this will need to be an iterative approach with an ongoing dialogue between preclinical work and clinical trials, and at the same time avoiding the temptation to short circuit this approach, with premature claims and commercialisation, all of which has the potential to derail this whole field.

Word Count: 3865

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TABLE 1; CLINICAL TRIALS USING CELL THERAPIES OR GROWTH FACTORS IN PATIENTS WITH DIFFERENT NEURODEGENERATIVE DISORDERS (NDDS)

NEURODEGENRATIVE DISEASE STATE	USE OF NEUROTROPHIC FACTORS IN CLINCIAL TRIALS	USE OF CELL TRANSPLANTS IN CLINCIAL TRIALS
ALZHEIMERS DISEASE	YES • NGF	NO
PARKINSON'S DISEASE	YES • NGF • GDNF/NTN	YES • Human fetal VM tissue • Adrenal medulla • Carotid body • Retinal pigmentary epithelial cells (Spheramine ®)
ALS	YES • CNTF • BDNF • IGF1	ONGOING • [NPC delivering GDNF)
HUNTINGTON'S DISEASE	YES • CNTF	YES • Human fetal striatal tissue
OTHER		

FIGURE 1 STRATEGIES TO REPAIR THE DISEASED BRAIN

In the healthy brain, the neurons project and synapse with target neurons. These axons are supported by myelinating oligodendrocytes and the astrocytes which form a syncytium that helps protect neurons and maintain the blood brain barrier (BBB). In disease states, such as that seen with NDDs, the neurons become dysfunctional and then die and this is often accompanied with a glial reaction and a change in the ECM. Treatments to thus repair and restore the brain in such conditions could include: (i) rescuing cells using neurotrophic factors; (ii) cell transplants to replace lost cells or support those targeted by the disease process; (iii) recruitment of endogenous cells to replace those lost to the disease process; (iv) modify the glial/inflammatory response and/or (v)modify the ECM including peri-neuronal nets.

FIGURE 2; CELL TRANSPLANT STRATEGIES

Cell grafts can be used to;

(i) replace lost cells and/or release factors locally so that they work in a paracrine fashion;
(ii) support neurons by improving the milieu around them, typically glial transplants;
(iii) repair local networks through short axonal projections in target structures;
(iv) repair long distance networks through long axonal projections to structures connected to the grafted target;
(v) to provide a substrate for axonal growth to promote repair.

Typically transplants being used to repair long distance projecting networks are placed homotopically whilst those designed to repair local networks are more likely to be heterotopically placed.

FIGURE 3; STRATEGIES FOR IN SITU REPAIR

This can be done through the; (a) recruitment of new neurons from endogeneous precursors originating from sites of adult neurogenesis or alternatively (b) through directed reprogramming of resident non-neuronal, glial cells